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Abstract

According to the data obtained in this experiment by means of the geldiffusion technique, the specific antigen was not detected in MH134 ascitic tumor, comparing the anti-C3H liver sera with anti-MH 134 tumor sera, though a loss of organ specific antigen and weak antigenicity were found in MH134 tumor extract. In order to detect some qualitative alteration, supposedly a gain in antigenic components, the transplant rejection test was carried out. The result of this test indicates that the relative not absolute resistance could be induced to C3H mice by this prior sensitization with cell free active extract eluted from MH134 tumor tissue by Fluorocarbon treatment. During these experiments, it became clear that MH134 ascitic tumor cell has weak immunizing properties so that prolonged lapse of time and large dose of antigen are inevitably necessary. Moreover, through Fluorocarbon treatment of the tumor homogenate, the cellfree, serologically active antigen could be obtained, which will serve well for the induction of the isologous immunization.

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IMMUNOCHEMICAL ANALYSIS OF ANTIGENIC PROPERTIES OF MH134 ASCITIC HEPATOMA

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It is known[21] that the tumor cell contains various kinds of antigens such as species-specificity, organ-specificity, blood group substances, histocompatibility factors, and heterophile antigens of Forssman type. Antigens of various animal tumors are studied by means of several immunological reactions, such as precipitation, complement fixation, and anaphylaxis. In filtrable tumors some investigators have successfully demonstrated the tumor specific antigenic substances besides the virus antigen. In nonfiltable tumors, however, it is still disputable whether the tumors do contain any specific antigens which are absent in the tissue of the normal organism.

The present investigation is concerned with demonstration of antigenic components of MH134 ascitic hepatoma cells compared with antigenic components of healthy C3H liver cells from which MH134 ascites tumor was originally derived. With a view to demonstrate these antigenic components one group of rabbits was immunized with extracts from MH134 ascitic tumor cells and another group was with extracts from C3H liver cells. Immune rabbit-sera were compared with each other by gel-diffusion technique in addition to absorption procedures with various homogenates. Moreover, to compensate the methodical deficiencies of this technique, the transplant rejection test was applied on this experiment in an attempt to verify the existence of antigen specific to MH 134 ascitic tumor.

As the result, the tumor specific antigen was not possible to detect in any appreciable quantity from MH134 ascitic tumor cell extracts by gel-diffusion technique. However, the MH134 ascitic cancer cell proved to show a lowered antigenicity together with loss of an antigenic component compared with those of healthy C3H liver cells. The antigen obliterated in MH134 tumor cells is supposed to be organ-specific. As for the reason of the difficulty in detecting the specific tumor antigen, it is assumed that the cancer cells have weak immunizing properties associased with its poor solubility so that it takes a longer period of time and larger doses of antigens for rabbit to be immunized, compared with the case of C3H liver cells. Though this method failed to detect the MH 134

tumor specific antigen, it is reasonable to think that a gain or qualitative alternation of antigenic components is involved by the malignancy. According to the transplant rejection test carried subsequently, inbred strain mice of C3H sensitized with Fluorocarbon-treated MH134 tumor extract prior to inoculaion of viable MH134 ascites cells showed resistance to the challenge of subsequent inoculation of those cells, so that it is supposed that there exists a specific antigen in the MH134 tumor cells, distinct from that of original C3H liver cells. This does not necessarily mean to be so-called MH134 tumor specific antigen. Moreover, isoantigen developed by repeated transplantations and residual heterozygotes of the inbred strain might play a role of inducing the resistance in pretreated C3H mice. However, these scanty data do not warrant the conclusion on this point. Such studies along this line are still in progress.

MATEIALS AND METHODS

Mice, inbred C3H (Kyoto) were purchased from The Mouse Colony, University Okayama. They weighed about 18～22 g at the start of experiments.

A strain of the MH134 ascitic hepatoma (MH134, in the 298th generat.) was obtained by courtesy or Dr. MATSUMOTO, Takeda Laboratory, Osaka. The tumor was kept in C3H mice of serial transfers. Transfers were done at weekly intervals by i. p. inoculations of 5×10^6 washed tumor cells. Tumor antigen was prepared from the MH 134 ascitic tumor homogenizing of fresh material in buffered physiological saline (pH 7.5). The comparative study was carried out between antigenic components of the MH 134 tumor cells and healthy C3H liver tissue from which MH134 ascitic hepatoma was originally derived. Antigens were prepared either as whole homogenate or as the supernatant fluid obtained by centrifugation of the homogenate at 3,000 r.p.m. Blood for control serum was withdrawn from rabbits before injection of antigens. The antigen mixed with Freund's adjuvant was injected into the two injection sites on the back of rabbit; 6 rabbits were sensitized with the same tumor extract. Freund's adjuvant was composed of the vigorous mixture of 1 ml of mineral oil, 0.5 ml of Arlacel A and 10 mg of dried, heat killed mycobacteria to 1 ml of antigen solution (1.0 mg N/ml). One week later, another injection of antigen emulsified with above mentioned Freund's adjuvant was administered. Thereafter, intravenous injection of antigen solution were repeatedly applied to the rabbits as shown in Table 1. The total of the antigen-nitrogen amounts to 10mg～15mg. The blood was withdrawn at varying intervals thereafter for the detection of precipitate antibody against the applied tumor extract. Ring tests were done with the immunesera withdrawn, which were clarified by centrifugation at 3,000 r.p.m. for 30 min. The test was carried out in small test tubes

Table 1 Immunization Procedure & Precipitate Reaction of Immune Rabbit Serum against C3H Mice Liver Cell Extract

Day	C3H mouse liver extract N mg	Route of injection	Date of bleeding
1	1.0	I. M. (F. A)	Bleeding
7	1.0	I. M. (F. A)	
18	2.0	I. V.	
20	1.0	I. V.	
21	1.0	I. V.	
22	1.0	I. V.	
24	1.0	I. V.	
25	1.0	I. V.	
35			Bleeding
37	1.0	I. V.	(precipitation (−))
38	1.0	I. V.	
39	1.5	I. V.	
40	1.0	I. V.	
41	1.0	I. V.	
51			Bleeding
52	1.5	I. V.	(precipitation (+))
53	1.5	I. V.	
60			Bleeding (precipitation (++))

(3×50mm), and a positive reaction usually occurred within a few minutes, when the precipitation amounted at least to 1.0γg of protein, showing a visible turbidity ring of antigen-antibody complex at the interface of both solutions. The preservation of the sera was achieved by freezing at −40°C, with adding the merthiolate in a proportion of one part per 10,000. For the purpose of analysis and identification of antigenic components, Ouchterlony's plate method[11] of gel-diffusion was employed. Preparation of diffusion plates was done as described in the previous report[18]. Before performance of the tests, quantitative determination of equivalence zones of this system was performed according to Heidelberger's method[4], for the purpose of obtaining equivalent antigen-antibody reaction on the agar plate. Then, a series of small wells, geometrically placed 1 cm apart from one another in a layer of gel on a plate, are filled with solution of diluted antigen solution (0.25 ml) and immunesera (0.25 ml) arranged to diffuse toward each other. Then precipitation was allowed to take place by storing the plate in a humidified 37°C incubator, empirically set 3 to 7 days. When the plate was fully developed, a drawing was made showing all the lines that developed and a photograph taken, then the plate was stored in a cage filled with formol gas. The antigenic solution used here was prepared by the

centrifugation sedimentation of 3,000 r.p.m. of homogenized C3H liver tissue and MHI 34 cancer tissue solution by grinding in Waring blender for 5 min and Potter's homogenizer for another 5 min at 4°C. Further purification of the antigenic solution was made by the Fluorocarbon treatment, (which did not cause any remarkable loss of antigenic components as far as precipitating tissue antigen is concerned as shown in previous report[18].) Antisera were absorbed by treating them with various dilutions of antigens (acetone powder of tissue homogenate) in test tubes for 1 hour at 37°C: the supernatant fluid by centrifugation at 30,000 r.p.m. represented the absorbed antisera.

The methods in the transplant rejection test are described as follows. In previous experiment, it was proved that the tumor specific antigen can be eluted and purified from tumor homogenate by means of Fluorocarbon treatment[18]. Therefore, 0.6 mg N/ml of the antigenic solution thus treated was subcutaneously injected divided two equal doses to inbred C3H mice, 6 mice in one group. The dose for this sensitization (0.6 mg N) corresponds to the extract eluted by Fluorocarbon treatment of 5×10^9 tumor cells. Three weeks after the last sensitization with the antigenic solution, viable MH134 ascitic tumor cells were subcutaneously inoculated in axillar region of the pretreated C3H mice in a dose of 1×10^5 tumor cells which is sufficient in the number of cells, capable of 100% "take" of the MH134 tumor transplant to inbred C3H mice according to the data of TAKEDA[19]. Another group of mice was inoculated with 5×10^6 tumor cells. Since the tumor cells inoculated, "take" of tumor and the size of tumor in diameter were measured and survival time of grafted mice was recorded in each group of mice with control.

RESULTS

Preparation of Rabbit Antiserum Against MH 134 Tumor Extract and C3H Liver Cell Extract: As mentioned in previous report, it took a longer period of time and larger dose of antigen for rabbit to produce a suitable antiserum against cancer cells than in the case of liver cells or serum. Therefore, a combination of Freund's adjuvant method with the intravenous method was employed for the preparation of the immune rabbit serum. The inoculation schedule performed was shown in Table 1. By the time of the 7th weekend of inoculation period suitable immuneserum were obtained. The total dose of antigenic nitrogen amounted to about 10 mgN. Two out of 6 rabbits were well immunized to this inoculation. Others did not respond so well to this inoculation. On the other hand, in the case of inoculation with MH134 tumor cell extract, as shown in Table 2, the response of rabbit to this inoculation was not sufficient by the time when the rabbit responded to C3H liver cells. It took over twice

Table 3 Immunization Procedure & Precipitate Reaction of Immune Rabbit Serum against MH 134 Tumor Cell Extract

Day	MH134 tumor cell extract mg N	Route of injection	Date of bleeding
1	1.5	I. M.	Bleeding
7	1.5	I. M.	
10	0.6	I. V.	
11	1.2	I. V.	
20	1.0	I. V.	
21	1.0	I. V.	
25	1.5	I. V.	
27	1.0	I. V.	
30	1.5	I. V.	
35	1.0	I. V.	
45			Bleeding
49	0.6	I. V.	
50	1.2	I. V.	
60			Bleeding
62	0.3	I. V.	(precipitation (−))
63	0.3	I. V.	
65	0.6	I. V.	
89			Bleeding
94	0.6	I. V.	(precipitation (±))
95	1.2	I. V.	
99	0.6	I. V.	
100	0.6	I. V.	
108			Bleeding
109	0.4	I. V.	(precipitation (±))
110	0.4	I. V.	
111	1.0	I. V.	
120			Bleeding
			(precipitation (+))

the period as compared with that of C3H liver cells. Even at the 18th weedend, blood withdrawn from 3 of the 6 rabbits immunized showed no efficient response to homologous tissue extract. Average dose of antigenic nitrogen was about 15 mg. In order to detect the antigenic difference between MH 134 tumor and original C3H liver cells, these two were compared by gel-diffusion test on agar plate, the results of which were described in text.

Analysis of the Antigenic Properties of MH 134 Extract compared with C3H Liver Antigen by Cel-Diffusion Technique: The antigenic components of C3H liver cell may be divided into several classes. General component

which is found in all the tissues of C3H mouse, so-called species-specific antigen, organ specific antigen, histocompatibility antigen, and heterogenous antigen. In fact, the immune rabbit sera against C3H liver cells reacted with the homologous antigen showing the several remarkable precipitating bands on the agar plates (Fig. 1). This precipitin reaction was consisted of 3 main bands and a few

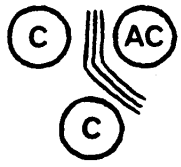

Fig. 1 AC=Anti-C3H liver serum C=C3H liver extract

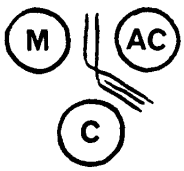

Fig. 2 M=MH134 tumor extract

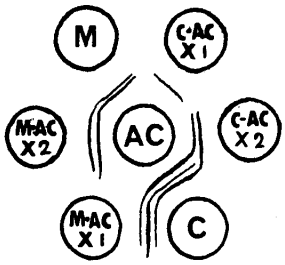

Fig. 3 C-AC×1=C3H liver extract absorbed with equivalent anti-C3H liver serum
M-AC×2=MH134 tumor extract absorbed with 1/2 equivalent anti-C3H liver serum

other weak lines, nearby the main bands, which did not always appear. These precipitin bands mean that three main antigenic components are contained in C3H liver cell. In contrast, the immuneserum against MH134 tumor cells reacted with the homologous antigen showing not any remarkable precipitation bands on the agar plates, as compared with those of C3H liver cell. This reaction was consisted of 2 main bands. These two bands seem to be common in those of C3H liver cell antigenic components by the fact that these lines are continuous with each other (Fig. 2). As far as this experiment is concerned, the tumor specific precipitin band was not detected in precipitin reactions on the gel-diffusion plate, though depletion of one antigenic component has been demonstrated in the gel-diffusion. Fig. 3 shows the precipitin reactions anti-C3H liver serum absorbed by various dilutions of MH134 tumor antigen and homologous C3H liver antigen. The anti-C3H liver serum in the central well reacted with both C3H liver antigen and MH 134 tumor antigen, producing continuous 2 chevron-shaped lines, and one additional line only at the side on C3H liver antigen. This indicates the presence of two common antigenic components, and one component that is present in healthy C3H liver cell, but not present in MH 134 tumor cell. By adding the equivalent amount of C3H liver antigen, one precipitation line located in between two main lines, which is absent in MH 134 cancer antigen, was absorbed and disappeared on the plate. Whereas two other lines,

which are common in MH134 and C3H, have barely persisted to absorption procedure with this amount of C3H liver antigen. The antigenic components of MH134 might have immunologically, remarkably weak antigenicity compared on the same nitaogen amount with those of C3H liver cells. It is assumed that its antigenicity will be less than one third that of C3H liver cell antigen. However, the qualitative difference in antigenic patterns between NH134 cancer cells and original C3H liver cells could hardly be detected by means of the gel-diffusion technique. The precipitin reactions between the anti-C3H liver serum and various kinds of extracts from C3H mice organs such as spleen, kidney, and serum were tried. Not any conclusive data were obtained in these comparisons except for vague cross-reactions present in larger parts of precipitating bands elicited by C3H liver extract, but a slight cross-reaction coincident to the band which was absent in MH134 tumor extract. According to these results, the antigenic component, which is absent in MH134 cancer, will probably be organ-specific antigen. Others belong to heteroantigens, and histocompatibility antigens. In no case was it possible to remove all the C3H liver antigenic components from anti-C3H serum by the absorption procedure with MH 134 extract. The middle precipitating line supposed to be due to the organ specificity was left unabsorbed even with inoculation with excess MHI 34 antigen (Fig. 4). The Fluorocarbon-treated MH134 tissue antigen and original crude MH134 antigen were tested by serological reagent of anti-MH134 rabbit serum. In this test, not any variation was found in the numbers of components visualized on a gel-diffusion plate. Therefore, the extract eluted by Fluorocarbon-treatment was supposed to be adequate for the use as cell free, serologically active material in the next experiment.

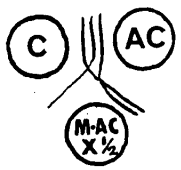

Fig. 4 M-AC$\times 1/2$=MH134 tumor extract absorbed with two fold equivalent anti-C3H liver serum

Attempts at Detecting MH134 Cancer Specific Antigen by Means of the Transplant Rejection Test: One group of six mice, 3 weeks after the sensitization with Fluorocarbon-treated antigen 0.6mg N, was challenged with subcutanous inoculation of 1×10^5 MH134 tumor cells. Another group was challenged with 5×10^6 MH134 tumor cells. Five out of 6 mice challenged with 1×10^5 MH134 cells showed the development of palpable tumor at the tumor injection site by 10 days after inoculation; mean size of tumor at that time being 0.7×0.7. In the following 10～14 days the tumor has shrinked and finally disappeared in 20～35 days after the tumor graft. One of them did not show any formation of tumor mass. Two of them died from unknown cause at the 32nd day and 48th day while tumor still remained. All the mice of other group,

inoculated with 5×10^6 tumor cells, were free of palpable tumor till the 7th day. However, in the following 7 days a palpable tumor had developed at the site of tumor injection with mean size of 1.2×1.2 and finally all the mice expired after various survival times. Those mice that remained healthy, gradually gained body weight as time passed, as shown in Fig. 5. The mice of control group with inoculation of 5×10^6 showed development of palpable tumor at the site of the tumor injection, by the 7th day after tumor graft; mean size of tumor at that time being 1.2×1.2. These tumor-grafted mice have rapidly gained their body weight for the first 10 days, along with the growth of inoculated tumor. Thereafter they have gradually lost their body weight and finally expired as shown in Fig. 5. Average survival time of control mice was 37 days. Next,

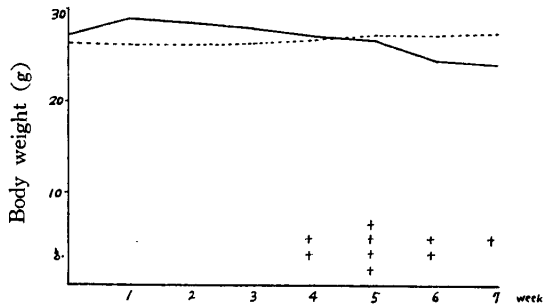

Fig. 5 Body Weight & Survival Period of Inoculated Mice
+ indicate "expire" of control mouse
—— nontreated C3H inoculated with 5×10 cells
- - - pretreated C3H inoculated with 1×10 cells

those mice that survived after rejection of the grafted tumor or without any signs of tumor growth were challenged again with 5×10^6 MH134 tumor cells. Some of them developed MH134 tumor within usual latent period. Not any measurable prolongation of survival in other mice rechallenged was observed in the present experiment. Average survival time was 41 days.

DISCUSSION

The techniques of tissue homogenization and differential centrifugation are now widely used. However, ascitic tumor cells, such as MH134 cancer cells show tendency to resist successful homogenization by the methods in common use, since the membrance and matrix of these cells are especially strong and can not be ruptured by procedures designed for liver cells[14]. This fact appreciably causes insolubility and irregularity of the tumor antigen coated with bulky mass of host tissue. Undoubtedly, a large part of this lag in the immunization may

be attributed to these characteristics of the tumor antigen used. In general, the magnitude of the rise of antibodies and the period of immunization for suitable antidody are reflected by the solubility of antigenic substance and its size related to the solubility and moreover, by the amounts and the numbers of antigen used. Consequently, a high concentration of suitable antibodies may well correspond to a high concentration of the soluble antigen used for immunization of animals. The lag of immunization period, found in the case of MH134 tumor as mentioned above, is supposedly due not only to this insolubility of antigen, but far more due to the fact that MH134 tumor tissue has scant amount and number of antigenic components. Therefore, it took almost twice the immunization time by the tumor antigen as compared with that by C3H liver antigen.

Substantial evidence has recently been provided to show the antigenic differences between the normal cell and its tumor. The causes underlying these differences may be quite different[2]. Filterable tumors contain certain viruses which penetrate into the cell from the outside and act as the etiologic factor of the tumors, hence they may be called as tumor viruses. In spite of varying degrees of association of the tumor viruses with normal cell components, these viruses behave as antigenic foreign to the animal in which they induce tumors.

Non-filterable tumors can be classified into spontaneous tumor, induced tumor, and transplanted tumors. In the present study, the antigen of transplated MH134 tumor was analysed by the gel-diffusion technique, while comparing antibodies of immune rabbit sera against the MH134 tumor tissue with antibodies of immune rabbit sera against C3H liver cells from which the tumor was originally derived. The study of antigens of transplantable tumors of any origin involves some difficulties associated with the genotype differences between the donor of the tumor and the recipient[1]. Most conclusive are experiments carried out on inbred animals although in these cases prolonged passage of the tumor might induce some modifications of its antigenic structure as detailed later. The author failed to detect any specific antigen from MH134 ascites tumor by means of the gel-diffusion. However, it is still premature to conclude by this result that there is no specific tumor antigen in the MH134 tumor cell distinct from C3H liver cell. As previously mentioned, the difficulty involved in the detection of specific tumor antigen may be due to less numbers of these antigens among the main bulk of tumor proteins indistinguishable from the antigen of normal tissue. Probably the scarcity of specific antigens in tumor tissue associated with poor solubility of them are also related to this difficulty involved in the detection of them. In the gel-diffusion technique applied in this experiment, the antigens should be soluble and diffusable in an agar layer. This is a serious limitation in the analysis of tumor antigens by such serological method as gel-diffuaion technique[6].

As ZILBER's experiment suggests the tumor antigen has generally a complex structure. The major part of produced antibodies in rabbits immunized by the MH134 tumor tissue may belong to heteroantibodies against species specific antigen and others, but not against organ specific antigen. As observed in the present experiment, the antibody elicited by the organ specific antigen of C3H liver did not react to MH134 tumor tissue extract. The metabolic disturbances associated with the carcinogenic process may result in the loss of some antigens from normal cells and peculiar simplification of their antigenic composition[20]. The absence of organ specific antigen in MH134 tumor tissue determined in present study, is connected to this supposition. SELIGMAN[21] has noted the simplification of the antigenic structure in animal tumors. WEILER and VOGT[20] have also found that the antigen is lost in liver cancer of rats induced by feeding with 4-dimethyl aminoazobenzene and localized this antigen in the membrane of endoplasmic reticulum. As the cells become malignant, a loss of some antigens contained in normal cells may ensue[10,16]. Recently, FRIEDRICH-FRESKA[3] et al. have succeeded in purifying this tissue antigen of normal rat liver microsomes, which is absent in induced liver cancer.

Now the question arises; Is there really any specific antigen in transplantable mouse tumor as a gain involved by malignant change? According to some investigators[21], the specific antigen is to be found not only in the transplantable hepatoma but also in that primarily induced by orthoaminoazotoluene. LEVINA[7] also has revealed in the hepatoma of mice an antigen which is absent from healthy liver, by means of anaphylaxis following desensitization. In MACULLA's[21] experiments, the nucleoprotein fractions prepared after MINSKY and POLLISTER (1942) from various transplantable mice tumors proved immunologically different from those of normal mouse organs. This difference was found in a strictly standardized test of complement fixation by the sera obtained through immunization of rabbits with various preparations from the tumor. However, these results are not completely accepted with regard to the isoantigenicity between the donor and the recipient of tumor. The alcohol extracts of various mouse tumors obtained by other investigators[2] are also antigenic substances, which are specifically reacting components common to various mouse tumors. From EHRLICH ascites mouse carcinoma and YOSHIDA ascitic rat sarcoma, an antigen of a lipid character has been isolated by LUND[21]. The nature of antigens detected in these studies is by no means certain. As a conclusion, the preparations produced by a similar may from different transplantable tumors and tested in different serological reactions naturally do not yield consistant results. The antigens of cancerous and embryonic tissues were supposedly found to be closely related in various transplantable mice tumors, especially similar to those of embryonic liver although not all of them possessed adult liver components[21].

MALMGREN[9] stated that in serological analysis of microsomes from mouse hepatoma and normal liver, quantitative but no tumor specific differences were observed. Consequently, it is reasonably supposed that MH134 tumor antigen is closely related to original C3H liver tissue antigen, so that the differentiation of these antigens is a difficult problem. In the present study by the gel-diffusion technique no antigan has been detected, that should be allocated only to the MH134 tumor cells, although a quantitative difference was noted in the reactivity between the preparations from normal C3H liver tissue and that from MH134 tumor, with loss of organ specificity of C3H liver. Therefore, the specific MH134 tumor antigen was viewed from the point of transplantation rejection which would occur in pretreated C3H mice with homologous cell-free, serologically-active extracts of MH134 tumor tissue. Formerly[17], it was not expected that isologous immunity might be detected in such fully isologous system as C3H liver cell and MH134 ascitic tumor. Recently, many investigators[12] have reported the resistance that could be built up in autochthonous and isologous various induced tumors and transplantable tumors. In such experiments for demonstrating the host resistance, the animals pretreated usually with heavy irradiated antigenic solution[6,13], were subsequently challenged with viable homologous tumor cells. Whereas in the present experiment, Fluorocarbon treatment was used instead of heavy irradiation. As in previous studies[18], it is evident that this treatment does not cause any measurable change in antigenic properties in as far as the gel-diffusion is concerned. Namely, no qualitatjve difference in antigenic patterns before and after this treatment does occur. The mechnism of the treatment with Fluorocarbon is not clear but is supposed to purify the antigen in the following procedure. The aggregates of antigens (virus antigen) and a ballast protein of the tissue may be destroyed by treatment with a Fluorocarbon medium which released pure antigen particles 10～20 mμ not sedimenting upon prolonged centrifugation. According to LINDENMANN's[8] report, the removal of the bulk of the tumor allowed the balance between the tumor and host to shift in favor of the latter, and liberate otherwise inaccessible antigenic materials from the cells. By the treatment of Fluorocarbon, also such effects might be suggested on the tumor extracts. Four of the six C3H mice pretreated by MH134 tumor extracts (0.6 mg N)treated by Fluorocarbon resisted subsequent challenge by 1×10^5 tumor cells in various degrees. Fluorocarbon-treated MH134 ascitic tumor tissue extract can induce a state of resistance in isologous C3H mice of the highly inbred strain from which MH134 ascitic tumor was originally derived. With the challenge of 5×10^6 tumor cells, all those pretreated mice ultimately expired though moderate retardation in latent period of "take" and prolonged survival time were demonstrated. Resistance was relative rather than absolute and broken down the dose of viable cell was increased. Although a few cases of the control, C3H

mice pretreated with C3H liver extract eluted through Fluorocarbon treatment showed slight induced resistance, almost equal with nontreated control. According to KLEIN[5], even isologous mice pretreated irradiated normal tissue and subsequently challenged with viable tumor cells exhibited a moderate resistance, which is considered to be the result of a nonspecific bolstering of the specific immune response, provoked subsequently by the first challenge dose of viable sarcoma cells. This fact means that the immune rabbit sera against MH134 ascitic tumor contained the specific antibody to resist the graft or the growth of the tumor subcutaneously inoculated into the inbred C3H mice. The MH134 ascitic tumor thus has some specific antigen which is absent in original C3H mice so that the Fluorocarbon-treated MH134 tumor extract could induce a state of resistance in isologous mice of C3H strain. Next, those mice that survived after rejection of the grafted tumor were rechallenged by MH134 ascitic tumor cells expired even after a longer survival time than that of control. This fact probably means the consuming of the induced resistance factor by previous challenge of tumor cell. This experiment has been carried out on inbred animals so the results might not have been due to isoantigenic differences between the donor and the recipient of tumor. In such long-propagated tumor as this MH134 tumor, considerable genetic differential might be supposed to accumulate through mutational changes in both tumor and hosts[1], RÉVÉSZ[13], in his study, has described that the isoantigenic differences that developed between a line of inbred mice and its transplanted tumor, in serial passage through long periods of time play a role to induce the resistant state in isologous host by the pretreatment with irradiated tumor cells. Further, he has shown that no similar resistance could be induced against lymphomas of recent origin. Taking these into consideration, it is still premature to conclude that this rejection phenomenon of tumor transplanted to the isologous animal pretreated with cell-free, serologically-active extracts of homologous tumor tissue, is not attributed to mutation[15] of the tumor or host, namely, isoantigen, as caused by repeated transplantations or residual heterozygotes[14] of the inbred strain host but naturally attributed to antibodies called forth to this tumor by means of the previous treatment with the antigen solution. These still scanty data do not warrant the conclusion that antigen of the MH134 ascitic tumor, which is absent in healthy C3H liver cells, is contained in tumor cells. Further study on this tumor is necessary. Especially, additional control experiment is required. These studies are still in progress and will be the subject of a separate report.

SUMMARY

According to the data obtained in this experiment by means of the gel-

diffusion technique, the specific antigen was not detected in MH134 ascitic tumor, comparing the anti-C3H liver sera with anti-MH134 tumor sera, though a loss of organ specific antigen and weak antigenicity were found in MH134 tumor extract. In order to detect some qualitative alteration, supposedly a gain in antigenic components, the transplant rejection test was carried out. The result of this test indicates that the relative not absolute resistance could be induced to C3H mice by this prior sensitization with cell free active extract eluted from MH134 tumor tissue by Fluorocarbon treatment. During these experiments, it became clear that MH134 ascitic tumor cell has weak immunizing properties so that prolonged lapse of time and large dose of antigen are inevitably necessary. Moreover, through Fluorocarbon treatment of the tumor homogenate, the cell-free, serologically active antigen could be obtained, which will serve well for the induction of the isologous immunization.

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REFERENCES

1. BARRETT, M. K., and DERINGER, M. K.: An induced adaptation in a transplantable tumor of mice. *J. Nat. Cancer Inst.* 11, 51～59, 1950
2. BUNTING, H.: Serological studies of transplantable mouse tumor. 15091a. *Yale J. Biol. & Med.* 15, 557～63, 1942
3. FRIEDRICH-FRESKA, H., SÜSS, R., LANKA, E., and BÖRNER, P.: Purification of an antigen of normal rat-liver microsomes disappearing in experimental liver cancer. Cellular control mechanisms and cancer: *Elsevier Press*, 272～78, 1964
4. HEIDELBERGER, M., and KENDALL, F. K.: A quantitative study of the precipitin rcaction between type III Pneumococcus polysaccharide and purified homologous antiboby. *J. exp. Med.* 50, 809, 1929
5. KLEIN, G., SJÖRGREN, H. O., KLEIN, E., and HELLSTRÖM, K. E.: Demonstration of resistance against methylcholanthrene-induced sarcomas in the primary authochthonous host. *Cancer Res.* 20, 1561～72, 1960
6. KORNGOLD, L., and PRESSM N, D.: The localization of rat lymphosarcoma antibodies in Murphy lymphosarcoma. *Cancer Res.* I4, 96～99, 1954
7. LEVINA, D. M.: Voprosy patogenesa immunol. Opukholei (The problems of pathogenesis and tumor immunology symposium) 127～50, 1964
8. LINDENMANN, J.: Immunity to transplantable tumors following viral oncolysis. *J. of Imm unol.* 92:6, 912～19, 1964
9. MALMGREN, R. A., BENNISON, B. E., ANDERSON, B. F., and RISLEY, C. C.: A serologic study of the microsome fraction of normal and neoplastic mouse tissues. *J. Nat. Cancer Inst.* 11, 1277～86, 1951
10. NAIRN, R. C., FOTHERGILL, J. E., and MCENTEGART, M. G.: Loss of gastro-intestinal

antigen in neoplasia. *Brit. med. J.* 30, 1791~93, 1962
11. OUCHTERLONY, O.: Antigen-antibody reactions in gel; IV types of reactions in coordinated systems of diffusion. *Acta path. & microbiol. Scand.* 32, 231~40, 1953
12. PREHN, R. T., and MAIN, J. M.: Immunity to methylcholanthrene-induced sarcomas. *J. Nat. Cancer Inst.* 18, 769~78, 1957
13. RÉVÉSZ, L.: Detection of antigenic differences in isologous test tumor systems by pretreatment with heavily irradiated tumor cells. *Cancer Res.* 20, 443~51, 1960
14. SAUER, L. A., MARTIN, A. P., and STOTZ, E.: Cytochemical fractionation of Lettré-Ehrlich ascites tumor. *Cancer Res.* 20, 251~58, 1960
15. SNELL, G. D.: Methods for the study of histocompatibility genes. *J. Genet.* 49, 87~103, 1948
16. STEPHANIE, H. B.: Comparison of antigenic components in normal and neoplastic tissues. *Exp. Cell Res.* 27, 584~52, 1962
17. SUGIURA, K., and BENEDICT, S. R.: *Amer. J. Cancer* 15, 2727~44, 1931
18. TAI, C.: Immuonchemical analysis of antigenic properties of human stomach cancer. *Acta Med. Okayama* 19:1, 1965 (in press)
19. TAKEDA, K.: Immunogenic study in antitumor antibody production against transplantable tumors; Part III. Immunogenic study in tumor-bearing mice treated with anticancer drugs and in mice immunized with heavily irradiated tumor cells. *J. Okayama Med. Assoc.* 75, 103~14, 1963 (in Japanese)
20. WEILER, E., and VOGT, P. K.: *Z. Naturforsch.* 15b, 213~21, 1960
21. ZILBER, L. A.: Specific tumor antigens. *Advanc. Cancer Res.* Academic Press 5, 291~329, 1958